

Biofilm adhesion (Crystal Violet assay)

Warning: Crystal Violet is a suspected carcinogen. Crystal Violet will stain your skin or clothes by contact and cannot easily be removed. Use gloves at all times when handling Crystal Violet and work in fume hood.

With this protocol you can compare adhesion ability/biofilm forming ability of isolated clones/mutants. The adhesion assay can be used as the first screening of a series of strains before investigating the most interesting ones in more details in our flow-chamber system.

Solutions:

0.1 % Crystal violet solution (0.1 g Crystal violet in 100 mL demineralized water)

0.9 % NaCl

96 % Ethanol

H₂O

Materials:

Use suitable microtiter plates. U-bottom or flat-bottom are suited.

Multipipette 300 µL

Plate reader (Measure at 585 nm or similar)

Day 1:

Inoculate strains you want to analyze in 10 ml test tubes. Use dilute or minimal media to avoid excessive growth.

Day 2:

Inoculate microtiter plate with the different strains of interest. Include blank e.g. 4 wells with media

1. Dispense 150 µL of ON culture in microtiter wells. If possible min. of 4 replicates is needed since large variation occurs.
2. Place the inoculated microtiter plate in a thick plastic bag and incubate over night at 37°C or 30°C (depending on strain etc., also, some clinical isolates needs two/tree days' incubation).
3. Alternatively, single colonies from plates can be inoculated by a tooth pick into the microtiter plate containing 150 µL media

Day 3:

4. After incubation measure OD₆₀₀ (or similar wavelength)
5. Empty all the wells by simply throwing out the liquid in a clinical waste bag (without using a pipette).
6. Transfer pre-warmed (37°C or 30°C) medium or 0.9% NaCl to a sterile Petri dish and use a multi-pipette to gently add 200 µl (avoid overloading) medium/NaCl to each well, and discard it by throwing. Repeat this washing step twice.
7. Add 200 µl (avoid overloading) 0.1 % Crystal Violet solution to each well (**use gloves and work in fume hood**) and let stand at room temperature for 15-20 min.

8. Discard the Crystal Violet solution by throwing out the liquid in crystal violet waste boxes (**placed in fume hood. Wear gloves**).
9. Wash three times with water (3x200 µl (avoid overloading)). Again discard by throwing out liquid in Crystal violet waste boxes.
10. Add 200 µl (avoid overloading) 96% ethanol; pipette up and down thoroughly in order to dissolve the Crystal Violet (use multi-pipette).
11. Read the plate in a plate reader using an excitation of 585 nm (or similar)
12. Gather results in spreadsheet taking into account the growth of individual clones by calculating the ratio of A_{600} (growth) versus A_{585} (crystal violet).
13. Leave the plate overnight in the fume hood for the liquid to evaporate and discard of plate in brown waste bags (biological waste)

Reference:

Reisner A, Krogfelt KA, Klein BM et al (2006) In vitro biofilm formation of commensal and pathogenic *Escherichia coli* strains: impact of environmental and genetic factors. *J Bacteriol* 188: 3572-3581